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Purification and comparison of two toxic peptides (CSTX-1 and CSTX-2) in the venom of *Cupiennius salei* (Ctenidae)

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Purification and comparison of two toxic peptides (CSTX-1 and CSTX-2) in the venom of *Cupiennius salei* (Ctenidae). - It is proposed that CSTX-1 (a 74 amino acid toxin) is similar to CSTX-2 (a 61 amino acid toxin)and that the remaining 13 amino acids (mainly lysine) cause its much higher toxicity.

Key-words: Spider venom - peptid toxins - amino acid sequence.

Thirteen toxic peptides were obtained from the venom of the spider *Cupiennius salei* after a complex separation procedure by HPLC. The purity of the isolated peptides was controlled by SDS-PAGE. These peptides differ remarkably in concentration and molecular size (between 2,500 and12,000 Da) and represent different types of toxins. Only 5 toxins (CSTX-1, 4, 8, 9 and 13) have higher concentrations than 4 mg/ml venom (Kuhn-Nentwig *et al.* 1994).

The toxins also differ in toxicity. To quantify this, the *Protophormia* biotest was used, where the test substance is injected into blowflies (*Protophormia* spec., Calliphoridae, adults, 1 day old). Remarkable differences in toxicity covering a range of more than a factor of one hundred were measured. The most potent toxin is CSTX-1 and it represents 18 % of the total venom protein.

CSTX-1 consists of 74 amino acids and has a molecular mass of 8,352.6 Da. The 8 cysteines form 4 disulfide bridges and are responsible for the tertiary structure of the protein. Actually, nothing is known about the bridge arrangement between the cysteines. Experiments to determine this arrangement by treatment with trypsin (Heck *et al.* 1994) failed in the case of CSTX-1. Generally, the structure of most spider peptide toxins are defined by their dominant structural feature of disulfide linkages which cause that these molecules are conformationally bound and thereby partially protected from proteolytic activity (Saccomano *et al.* 1994).

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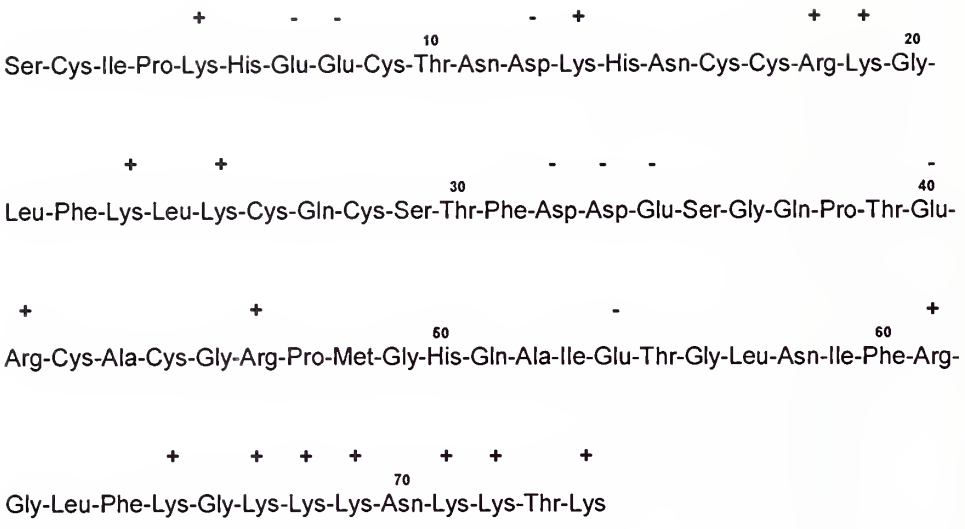

FIG. 1

Charge distribution in the amino acid sequence of CSTX-1.

Unusual in CSTX-1 are 7 lysines among the last 10 amino acids. A search in a data bank for protein sequence data showed that until now no protein with such an unusual sequence had been found. This lysine rich tail is also responsable for the distribution of the charges within the molecule so that a strongly positive section results, presumably important for the binding process at its target structure (Fig. 1). It is possible, that the toxicity of CSTX-1 is caused by or correlated to this lysine rich part. However, this could not be proved, because experiments to cut of this end by treatment with proteases such as carboxypeptidase P failed.

By checking 9 independent lines of monoclonal antibodies against CSTX-1 (Malli et al. in prep.) the obtained antibodies showed an additional specificity against CSTX-2 as shown in an immunoblot (Fig.2).The amino acid analysis and the theoretical calculation of the CSTX-2 show a high similarity with CSTX-1 when the last 13 amino acids are omitted (Tab.1). So we could estimate the molecular mass of CSTX-2 with 6,865.75 Da. Investigations by mass spectrometry (Fig. 3) confirmed this calculation with an estimated molecular mass of 6,864.5 Da and showed, that the molecular masses of CSTX-1 (minus the last 13 amino acids) and CSTX-2 are identical. A first amino acid sequence analysis with non-reduced and non-alkylated CSTX-2 could be performed until position 10. So far, it is identical with CSTX-1, two gaps in the sequence probably indicate the position of 2 cysteines (Fig.4).

We now conclude, that CSTX-2 is a shorter form of CSTX-1. Since CSTX-1 (LD50 0.41 µg/ fly) is 20 times more toxic than CSTX-2 (LD50 8.16 µg/fly) there is strong evidence, that the toxicity of CSTX-1 is mainly caused by its lysine rich

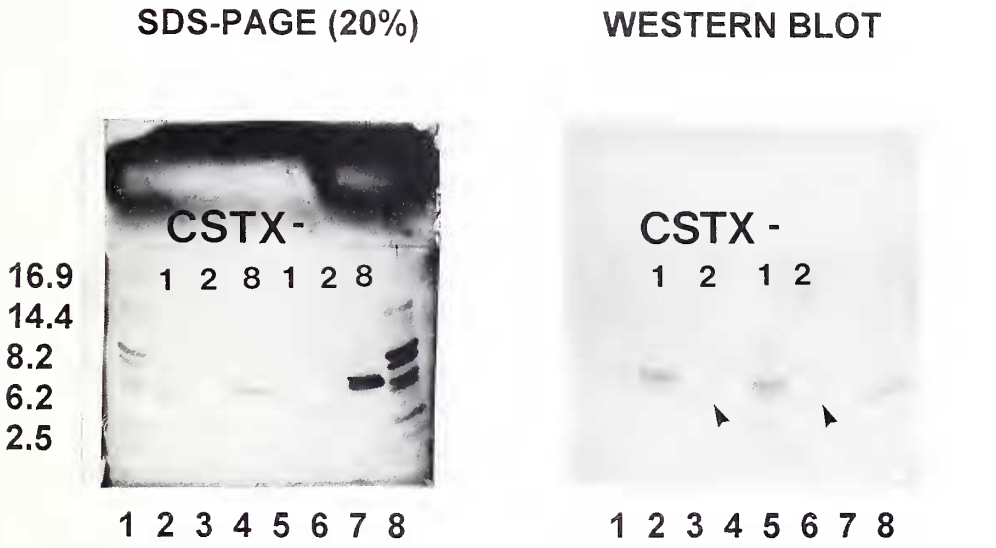

FIG. 2

Western blot analysis of CSTX-1 and CSTX-2.At the left side SDS-PAGE (20 % acrylamide, silver stained) of CSTX-1 (lane 2 & 5), CSTX-2 (lane 3 & 6) and CSTX-8 (lane 4 & 7). The concentrations of the probes are 4 µg for CSTX-1 & 2 and for CSTX-8 6 µg (lane 4) and 8 µg (lane 8). Lane 1 and 8 are molecular mass markers (2.5-17 kDa) (Pharmacia). At the right side is the immunoblot (Cellulosenitrate, 0.2 mm) of this SDS-PAGE with a monoclonal antibody against CSTX-1. Staining is caused by peroxidase-conjugated, goat anti-mouse immunoglobulins.

TAB. 1
Amino acid analyses of CSTX-2

AS	theoretical	M/M	(+/-)
ASP/ASN	6	6.2	0.2
GLU/GLN	8	8.6	0.6
SER	3	2.6	-0.4
GLY	5	5.3	0.3
HIS	3	3.4	0.4
ARG	4	4.2	0.2
THR	4	3.8	-0.2
ALA	2	2.1	0.1
PRO	3	3.1	0.1
TYR	0	0.1	0.1
VAL	0	0.1	0.1
MET	1	1	0
CYS	8	6.8	-1.2
ILE	3	2.8	-0.2
LEU	3	3.3	0.3
PHE	3	2.9	-0.1
LYS	5	5.4	0.4
TOTAL	61	61.7	0.7

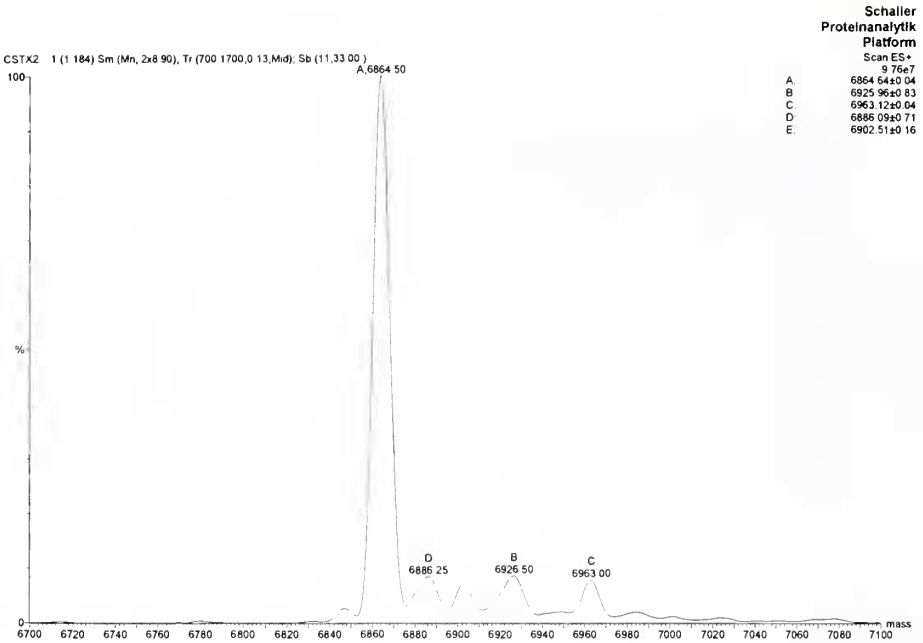

FIG. 3

Mass spectrometry (electrospray-MS) of CSTX-2 (5 pMol/µl).

CSTX-1 Ser-Cys-Ile-Pro-Lys-His-Glu-Glu-Cys-Thr-Asn-.....

CSTX-2 Ser-XXX-Ile-Pro-Lys-His-Glu-Glu-XXX-Thr

FIG. 4

Comparison of the N-terminal amino acid sequence of CSTX-1 (reduced and alkylated) and CSTX-2 (non-reduced).

carboxyl end. This could mean, that a positively charged domain is responsible for the binding to a target structure such as ion channels in the cell membranes of neurons.This is supposed by REILY *et al.* (1995) for ω-Aga-IVB, a toxic peptide from the spider *Agelenopsis aperta*. However, we do not know, whether such a shorter form of a toxin is the result of a degradation process induced by proteases or really reflects two independent toxins.

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